THE SPERMATHECA AND METHODS OF FERTILIZATION IN SOME AMERICAN NEWTS AND SALAMANDERS.

B. F. Kingsbury, Ph. D., Defiance, O.

In the proceedings of the American Microscopical Society for 1894, in a paper upon the "Histological Structure of the Enteron of *Necturus maculatus*," the writer alluded to the presence of "*Receptacula seminis*," in the dorsal wall of the cloaca of the female in this animal. Certain inconsistencies between the conditions in this one form, and statements made by investigators who had worked upon European salamanders alone, showed the desirability of a knowledge of the relations existing in a wider range of forms, and a study of the cloaca in the female of six species of American urodeles was undertaken.* These forms were *Necturus*, a perennibranchiate purely aquatic form; *Diemyctylus viridescens*, aquatic in its larval and adult state, but passing through a land stage; *Amblystoma punctatum*, a terrestrial salamander in the adult, except at the breeding season; *Desmognathus fusca* and *Spelerpes bilineatus*, forms which seem to adapt themselves readily to either a land or water existence; and *Plethodon erythronotus* and *glutinosus*, forms which are said to (Cope) pass no period, even as larvæ, of their existence in the water. These are representatives of five families and two orders of Amphibia, and present in variation of habit of life a good series from a purely aquatic to as purely a terrestrial existence.

The general result has been the recognition in all of structures in the cloacal wall of the female which serve as reservoirs in which the zoösperms of the male are received, functionally comparable, therefore, to the *Receptaculum seminis* of certain insects and other Arthropods. This term, however, by which they have been designated hitherto, is not strictly applicable to the

*This paper was prepared in the anatomical laboratory of Cornell University. I desire to express my appreciation of the abundant material and facilities placed at my disposal. At the suggestion of Professor Gage this investigation was made, and to his interest and advice is largely due what of value is herein contained.

organ as a whole, since in certain urodeles, *Necturus* for example, there is no unity of structure, there being many pouches or *receptacula*. Instead of *receptaculum seminis*, *spermatheca* is preferred as a euphonious mononym, and when there are many disconnected tubules which function as reservoirs for the zoösperms, each will be called a spermatheca. Therefore, in such forms many spermathecas would be recognized.

Strictly the ascertainment of the existence, state of development and structure of the spermathecas in the female would belong to an investigation of the development and life history of each of the forms here studied, and a discussion apart from a consideration or knowledge of the mode of mating, fertilization and ovulation is, in some respects, disadvantageous. Since, however, these related facts have been treated of somewhat monographically in the case of two of the species here studied, and either the presence of spermathecas has not been considered at all, or assumed to be the same as in the European forms in which it has been studied, the present research seems fully justified, especially as it is hoped that in the case of *Plethodon* and *Desmognathus* it may be but preliminary to the ascertainment of the mode of mating in these peculiarly interesting forms.

Although generalizations and distinctions of wide application should be made with caution, it appears to be a constant difference between the tailed and tailless forms of Amphibia, that in the former fertilization of the ovum is internal, in the latter external. Indeed, it is probable that in all the *Urodela* not only is fertilization internal, but it is accomplished by the same mode of mating. In all the forms so far studied it consists in a more or less complicated "courtship," which culminates in the deposition by the male of one or more spermatophores, consisting each of a gelatinous body bearing on its summit a mass of zoösperms. Over these the female passes, and the zoösperms are either actively grasped by the distended lips of the cloaca of the female, or cling to the outside and enter apparently of their own activity, independent of any efforts on the part of the female; there seems to be a difference in different forms in this respect. Within the cloaca of the female

they find their way into the spermathecas, and there remain until the time of ovulation. (See under *Salamandra atra* in Concluding Remarks.)

As in so many other matters of habit or anatomical detail in the Amphibia, it was in European forms that the mode of fertilization was first carefully observed, though a long time was required before the matter was at all understood. Spallanzani, in 1785, first showed that fertilization in the Triton was internal. He considered, however, that the zoösperms became diffused in the water and in that way entered the cloaca of the female, a view which later observations have shown to be incorrect. Rusconi, likewise, affirmed the internal fertilization of the ovum in the Triton, and that the male deposited the zoösperms externally, no true copulation taking place. With Spallanzani he believed that internal fertilization was accomplished by the diffusion of the zoösperms in the water, and that they thus obtained entrance to the cloaca of the female. He still further made the mistake of believing that an external fertilization took place in addition to the internal. Other writers also recognized the fact of internal fertilization in these forms, and the views of Spallanzani and Rusconi were commonly accepted. To my knowledge but one observer (Finger) affirmed that a true act of copulation took place in the Triton.

In 1858 appeared Siebold's significant discovery of the presence in *Salamandra atra* and *maculosa* and *Triton igneus*,* *cristatus*

* The species are here referred to under the names used by the various writers. Where different, the following sets forth their identity with the genera and species recognized by Cope:

GENERAL.	*COPE.*
SALAMANDRIDÆ.	
Triton cristatus.	Hemisalamandra cristata.
Triton alpestris.	Triturus alpestris.
Triton igneus.	Triturus alpestris.
Triton tæniatus.	Triturus vulgaris.
Triton abdominalis.	Triturus vulgaris.
Triton punctatus.	Triturus vulgaris.
Megapterna montana.	Triturus montanus.
PLEURODELIDÆ.	
Pleurodeles waltlii	Pleurodeles waltli.
Triton palmatus.	Diemyctylus palmatus.
Triton helveticus.	Diemyctylus palmatus.
Euproctus pyrenæus.	Diemyctylus asperus.

and *tæniatus* of groups of blind pouches in the dorsal wall of the cloaca in the female, filled with living zoösperms. This proved conclusively, if indeed further proof were necessary, that in these forms at least fertilization was internal. He was led by his discovery, however, to the erroneous belief that a direct sexual communication was therefore necessary.* Robin, '74, it appears, was the first to recognize the true mode of fertilization in the tailed Amphibia (Axolotl and *Triton alpestris*, *palmatus*, *cristatus* and *abdominalis*, or *punctatus*). By him the spermatophores were recognized and described as such. Gasco later, in 1880, also described the true mode of fertilization in the Triton, and the following year he was enabled to report the same mode of fertilization in the axolotl. The deposition of the spermatophores was described, and he stated that the zoösperms were actively taken up by the cloacal lips of the female in both forms. Zeller, '91, later states that this is not the case in Triton; Fick, however, confirms it for the axolotl. The mating habits of two European genera of the *Pleurodelidæ* and one genus of the *Salamandridæ*** were described by Bedriaga, and though his observations were fragmentary, were found to resemble quite closely that described by Gasco for the Triton. It remained for Zeller, '90, to gather together these more isolated observations, and supplement them in an interesting paper by observations of his own upon the mating habits of the genera *Triton*, *Salamandra*, *Axolotl*, *Pleurodeles* and *Diemyctylus*, in all of which occurs the same fundamental plan of fertilization, the preliminary courtship, the deposition of spermatophores by the male and the reception of the zoösperms by the cloaca of the female.

To the writer's knowledge only two American forms have been observed at all, and but one carefully, *Diemyctylus*. The mating

*By both Zeller and Jordan, '91, it has been noted how persistent was the influence of this opinion of Siebold's, so that in many standard text-books of zoölogy the statement persists that a copulation takes place. Cope further makes the unqualified statement that in tailed Amphibia a copulation occurs.

**_Pleurodeles Waltlii_, _Megapterna montana_ Savi, _Glossoleya Hagenmulleri_, and _Euproctus (Hemitriton) pyrenæus_.

of this newt was first observed by Zeller who speaks of it as *Triton viridescens*, later by Gage and by Jordan, '91, and the preliminary courtship, deposition of spermatophores and the reception of the zoösperms by the female are minutely described.

Observations upon the second American form, *Amblystoma punctatum*, have been very fragmentary, and Clark, by whom they were made, drew the conclusion that in this form fertilization was external, and is quoted to that effect by Balfour. There is little doubt but that what he observed was the deposition of a spermatophore* and that *Amblystoma* agrees with the other urodeles in its breeding habits; indeed, the thrice-repeated observations on the axolotl would leave nothing else to be expected. Were demonstration of internal fertilization necessary, it has twice been furnished in the anatomical laboratory of Cornell by the development of eggs laid by female *Amblystomas* with no male present. A study of the spermathecas also demonstrates the fact completely.**

Thus we see in all the forms so far observed*** a constant mode of internal fertilization occurs, and as stated by Zeller, '90, similar mating habits will probably be found in the remaining urodeles. On the spermathecas in which the zoösperms are stored very little has been said. Zeller refers to them merely without discussing their structure. Their presence has been

*Clark says, p. 106, "the males showed no inclination to clasp the females, but quietly deposited quite large masses of an apparently rather thick liquid, opaque white, on the bottom of the dish in which they were kept. Upon examination this liquid was found to consist of spermatozoa moving rapidly in a liquid. The eggs were found to have adhering to their outer shells, shortly after, a considerable number of these male elements, but I could not succeed after trying a great many times in finding any spermatozoa within even the outer shell. Most of the eggs were laid during the night, and by nine o'clock the next morning the first segmentation furrow had usually made its appearance."

**Eycleshymer, who has worked up the development of *Amblystoma*, evidently considered it unnecessary even to allude to the internal fertilization, despite the fact that published statements had been made to the effect that fertilization in this form was external.

***In *Proteus* the mere fact that fertilization is internal has been demonstrated by Chauvin, '83.

alluded to in *Salamandra*, *Triton*, *Diemyctylus*, *Geotriton* and the axolotl, but in *Triton* alone has their structure been considered in any detail.

THE CLOACA OF THE MALE AND ITS GLANDS.

It is not the purpose to discuss the structure of the cloaca and the glands belonging to it in the male, as it is but incidentally connected with this investigation. The attention I have given them is entirely superficial; indeed the complexity of the structure and the opportunities afforded in the glands to study the changes undergone by the secreting cells from a resting state to one of exhaustion, would render a special study productive of valuable results; such a study has been made by Heidenhain in *Triton cristatus*, *alpestris*, *tæniatus and helveticus*, especial attention being paid by him to one of the groups, the pelvic gland. In view of comparisons that have been made and will be made here between the cloacal glands of male and female, a few words must be devoted to the relations in the male.

Our knowledge of the cloaca is, as it is hardly necessary to say, based on the conditions in European forms, especially the Tritons, and chiefly through the writings of Rathke, Finger, Duvernoy, Leydig, Blanchard, and especially Heidenhain, who has devoted a most careful monograph to the structure of the cloaca and its glands in the Triton. As his description is the most complete it may be made the basis of the following statements on the conditions in that genus: Two portions of the cloaca are recognized by him, an ectal, caudal, ventral chamber, called by him the cloacal chamber, (Kloakenkammer) and an ental, more dorsal and cephalic tubular portion, the cloacal tube (Kloakenrohr). In the outer portion of the cloacal walls there is on each side a furrow which runs caudad and ectad (ventrad), and appearing upon the lips of the cloaca divides them into two limbs, an inner and outer; both the first unite to limit the vent caudad, while the latter do not unite, but end freely. In the depression occur (in Triton) about twenty thread-like papillæ at the summits of which are the orifices of the tubules which constitute the abdominal gland (Bauchdrüse).

The walls of the cloacal chamber are raised into ridges which run caudad and ventrad toward the lips of the cloaca. The cloacal cavity does not terminate roundly in its caudal end, but projects slightly caudad each side of the meson, constituting the posterior recesses. On the dorsal side of the cloacal chamber is a depression (Dorsalrinne) which proceeding cephalad becomes T-shaped and is the direct continuation of the tubular portion of the cloaca. The shape and indeed the depression itself is doubtless entirely obliterated when the cloaca is filled with feces. Ventrad of the depression and therefore truly from the ventral wall of the cloaca, though in appearance from the dorsal side, extends a tongue-like elevation whose shape and size appears to be altered by contraction.*

Dorsal and ventral ciliated areas are recognized, the former extending from the level of the caudal end of the kidneys (slightly caudad of the uro-genital papillæ) to the caudal end of the dorsal depression whose epithelium is ciliated throughout. The ventral area, according to Heidenhain, is more limited, extending from about the level of the cephalic end of the dorsal area to the caudal limit of the cloacal tube, *i. e.* slightly cephalad of the cephalic end of the cloacal opening. The remaining epithelium of the cloaca he found to be composed of a single layer of mucous cells which continued up to the edge of the cloacal lips where transition to the epidermis is rather rapid, although the cloacal epithelium adjacent to the epidermis is two layered. Therefore Heidenhain concludes that the epithelium of the cloaca is entodermal and considers the pelvic and cloacal glands as entodermal in origin, while the abdominal gland tubules opening upon papillæ which are covered with the stratified epithelium of the skin are ectodermal.

*To this had been applied by earlier writers the term penis. This is unfortunate; it has long been known that this is no copulatory organ. The name of cloacal papilla employed by Blanchard is also unsatisfactory and apt to introduce confusion. Heidenhain has not thought fit to introduce a new term, and since I only refer it to incidentally, it will not be necessary to do so here.

Before Heidenhain* but two glands were recognized as belonging to the cloaca of the male, the cloacal gland (Kloakendrüse, Leydig; Afterdrüse, Rathke) and the pelvic gland. Heidenhain has recognized another group of tubules included by former investigators (except Duvernoy) with the pelvic gland, but which he separates as the abdominal gland. The cloacal gland entirely surrounds the cloacal chamber and forms the basis of the cloacal wall, except on the dorsal side where the continuity of its mass is interrupted by the dorsal depression. Its tubules are quite straight, unbranched and open in most part into the cloacal chamber upon the summits of the longitudinal folds mentioned above. The pelvic gland occupies a position on the dorsal side of the cloaca. Its tubules open upon the dorsal ciliated area. When the dorsal depression has assumed the T-shape the openings are confined to the lateral walls of the depression, that is, to the stalk of the T; farther cephalad a few tubules open in the non-ciliated zone separating the dorsal and ventral ciliated areas. No openings occur in the ventral ciliated area. The tubules of the third gland newly recognized by him, the abdominal gland, open as stated above, upon the summit of the papillæ which are situated in the depression between the two limbs of the cloacal lip. From their openings the tubules run cephalad on each side, and form the larger part of the gland mass which is situated in the abdominal cavity between the abdominal muscles and the peritoneum. The tubules are in part forked at the end. From a histological consideration five forms of tubules were recognized, one which Heidenhain was inclined to regard as a special kind of gland. He was unable to determine in how far the others should have like recognition, or merely represented different phases of secretive activity of the same kind of gland.

Without discussing the value of the distinction into cloacal tube and cloacal chamber, or the entodermal or ectodermal nature of the epithelium and glands, the following brief observations and comparisons may be hazarded on the relations exist-

*Duvernoy, however, recognized two portions of the pelvic gland which he named *prostate abdominale* and *prostate pelvienne*.

ing in the males of the five genera studied in this investigation. *Diemyctylus* may be conveniently considered first, since it is the most nearly related to the Tritons and the relations at the cloaca approximate those in that form most closely. The three glands were readily recognized and their relations agreed closely with those in *Triton*. The dorsal depression was well developed and assumed the T-shape described for that form. The dorsal ciliated area was large and lined the dorsal depression throughout. It began as a mesal area and spread laterad as it progressed caudad until not only was the epithelium of the T-shaped depression ciliated, but also that adjacent to it in the dorsal wall of the cloaca. From the ventral wall an elevation projected slightly caudad of the uro-genital papillæ, which caudad became broken up into the ridges on which the cloacal gland opened. It was covered with ciliated epithelium which extended caudad upon the ridges spoken of above, almost to the cloacal lips. In the other four genera the ciliated areas were much as in *Diemyctylus*. In all, the ventral tract extended caudad upon the high ridges which bore the mouths of the cloacal gland tubules, being therefore much more extensive than in the *Triton*. The presence of ciliated areas in the male cloacas of purely terrestrial as well as purely aquatic urodeles, clearly disproves the view advanced by Leydig and quoted with favor by Hoffmann that a ciliated condition of the cloacal epithelium was to be associated with an aquatic life. It is a peculiar fact that no ciliæ were found in the cloaca of the female in any species examined save *Amblystoma* and *Plethodon glutinosus*, which will be spoken of subsequently.

The cloacal glands in *Diemyctylus* seemed to be much as in *Triton*. The pelvic gland tubules opened into the dorsal depression in four groups, viz., proceeding caudad, (a) a cephalic mesal group of short tubules whose mouths opened at the summits of low papillæ; (b) farther caudad lateral groups opening upon the stalk of the T; (c) a few tubules opened in the non-ciliated area ventrad of the dorsal ciliated tract; and (d) a mesal group opening on a small elevation in the caudal portion of the dorsal depression. The mouths of the tubules composing the cloacal

gland were situated as stated above in the summits of the ridges which radiated toward the cloacal lips where they were succeeded by large villi on which the tubules opened two and three together, around the cephalic end of the cloacal opening. The cloacal gland in all the genera presented much the same appearance, and the relation of the mouths of the tubules to the longitudinal ridges on the cloacal wall was constant in all. In *Necturus* and *Plethodon* these ridges were especially high and thin. In *Necturus*, *Desmognathus* and *Plethodon* the ridges were succeeded at the edge of the cloacal lips by papillæ which bore mouths of the tubules, though in the last two genera they were short. The tubules in the specimens examined were filled with a stringy secretion which took the hæmatoxylin stain. The cells appeared as figured by Heidenhain, reticulated and staining blue; undoubtedly, as stated by him, this gland is mucin-secreting.

The abdominal gland in *Diemyctylus* may be easily recognized and its relations are much as in *Triton*. As in that genus, its tubules open upon slender papillæ near the caudal end of the vent. From their ducts the tubules pass cephalad, laterad to the mass of the pelvic gland, to form with the pelvic gland a mass between the peritoneum and body muscles in the caudal end of the abdomen. In *Diemyctylus* the mass of its tubules extended ventrad to lie beneath the peritoneum dorsad of the pelvic arch. The free ends of the cells were filled with small globules of secretion which stained but lightly. In some cases almost the entire cell was filled with these globules, the nucleus surrounded by a scanty mass of protoplasm was cramped in the basal end of the cell. The papillæ on which the tubules opened were covered with a stratified epithelium which resembled the epidermis.

Necturus, in the configuration of the cloaca, resembled *Diemyctylus* closely. The dorsal depression, however, did not assume the T-shape as in that form. Both the cloacal and pelvic glands were greatly developed; a sufficiently careful study of the glands was not made to enable me to determine satisfactorily whether or not the abdominal gland was present. I consider it present though its tubules do not open from papillæ, nor do they

extend cephalad as in *Diemyctylus*, but are related more as in *Desmognathus*.

The cloacas of *Plethodon* and *Desmognathus* were much alike. Slightly caudad of the openings of the ureters there is a well marked elevation on the dorsal wall covered by ciliated epithelium which farther caudad breaks up into small papillæ bearing the mouths of pelvic gland tubules. Just cephalad of this are lateral grouped gland tubules which appear in *Plethodon*, *Desmognathus* and also *Amblystoma* to be of quite a different character from the other pelvic gland tubules, so it appears as if in these forms the pelvic gland were composed of two distinct kinds of tubules. The homolog of the abdominal gland could be recognized, but in both genera its tubules were short, being more developed in *Desmognathus* than in *Plethodon*. In the latter its tubules opened at the caudal end of the slit-like vent upon the epidermis covering the edges of the cloacal lips, but not upon papillæ. In the former the tubules open on the dorsal side of the cloaca near its caudal limit, upon papillæ, but clearly within the cloacal epithelium. These variations are of importance in considering the ectodermal or entodermal origin of the gland.

Of *Amblystoma* I need only mention in addition to what has already been said, that all three glands may be recognized; the cloacal lips are simple and do not possess the fringe of papillæ present in *Necturus* and *Diemyctylus*.

Formerly when attention was first called to these glands as accessory to the genital organs, it was attempted to homologize them with glands found in higher forms, and the pelvic was regarded as a prostate gland (hence the name applied by Duvernoy, and others.) Wiedersheim stated unqualifiedly that there can be no doubt but that these glands represent the prostate and gland of Cowper of higher forms.* There is little doubt now but that, as stated by Heidenhain and Zeller, these glands, opening upon the cloaca discharge a secretion which constitutes the body of the spermatophore, forming thus a base to give support on its

*"Dass diese Bildung der Prostata und den Cowper'schen Drüsen der höheren Wirbelthiere entspricht, kann wohl keinem Zweifel unterliegen."

summit to the zoösperms. To find, then, in all these genera that the same glands are present at the cloaca and well developed, is pretty strong circumstantial evidence that the same mode of fertilization occurs in all, though the deposition of spermatophores has been observed only in one and probably so in a second. I confidently believe that in the proper season of the year the terrestrial *Plethodon* will be found to go to the water for the purpose of mating just as does *Salamandra* of Europe, unless the spermatophores may be deposited with equal efficiency on land.

THE CLOACA OF THE FEMALE AND THE SPERMATHECA.

Less attention has been paid to the cloaca of the female than the male. This is perhaps because in the European Salamanders its structure is so much simpler. The following brief historical review will indicate how fragementary is the attention that has been bestowed.

Rathke ('20) and Leydig ('53) both recognized the presence of glands, doubtless the spermathecas, in the cloaca of the female *Salamandra*, though neither seems to have investigated the *Triton*. The latter described them as formed of cylindrical tubules of a caliber enlarging toward their end.

Siebold ('58) was the first to detect the presence of living zoösperms in these tubules, describing them in *Salamandra atra* and *maculosa* and *Triton igneus*, *cristatus* and *tæniatus*.

Wiedersheim ('75) merely alludes to the question of the presence of spermathecas in the genera *Salamandrina* and *Geotriton*, whose anatomy he made the subject of a monograph.*

Blanchard ('81) next mentions these in *Triton* (species not named) in connection with the pelvic gland of the male with which he regarded them as homologous though atrophied and function-

*Of *Salamandrina* he says "Die von Siebold entdeckten schlauchförmigen *Receptacula seminis* sind auch hier in zwei Gruppen vorhanden; jedoch gelang es mir nicht, in ihnen Zoospermien zu entdecken;" of *Geotriton*, "auch finde ich beim Weibchen keine Spur der Receptacula seminis, wohl aber frei in der Cloakenhöhle liegende Zoospermien, wie bei Salamandrina." He is therefore misquoted by Hoffman, '78, who states that he found in neither form a trace of spermathecas.

less as glands. He, however, did not agree with Siebold as to their functioning as spermathecas.

Heidenhain ('90) who so thoroughly investigated the cloaca of the male *Triton* discusses the female merely in connection with the discovery of the rudimentary tubuli which he regarded as representing the abdominal gland of the male.

Jordan, '91, speaks of finding in *Diemyctylus* zoösperms "in the ducts of two groups of gland-like structures situated in the cloacal wall just below the entrance of the oviducts." He discusses the problem of how the zoösperms become ensconced in their "snug resting places," and his views will be referred to subsequently.

Stieda, '91, furnishes us with a minute account of the cloaca and the spermathecas in the female *Triton*. His discussion is the most detailed of all, though he does not mention the relation of the spermathecas to fertilization, and he does not appear to have been fully familiar with the literature.

Fisher, '91, in a more general article upon the anatomy of *Geotriton fuscus* finds the "receptaculum" present as a single unpaired organ which he considers homologous with the pelvic gland of the male. No zoösperms were found in the gland examined by him. Wiedersheim's statement that there is no spermatheca in *Geotriton* is thus disproved.

Of the above writers, the accounts of Siebold and Stieda are most circumstantial. The salamandridæ have been by far the most carefully studied; while in the representatives of two other families (*Diemyctylus*, *Salamandrina* and *Geotriton*) little more than the mere presence of the spermathecas has been reported.

Before proceeding to a discussion of the genera examined by me it will be well to review briefly the structure and relations of the cloacal glands and spermathecas of the female as they have been ascertained to be in the family of the Salamandridæ. As compared with the cloaca of the male that of the female is quite simple; there are no representatives of the glands so greatly developed in the male except the comparatively simple spermathecas (if they are representatives) and in *Triton* the rudimentary

tubuli discovered by Heidenhain. The cloacal walls are equally simple and the configuration not so varied; well-marked dorsal and ventral folds exist with smaller lateral ones. The spermathecas are simple blind sacks lined with a single layer of cubical cells which seemed to Stieda, to have no secretory function; a circular layer of plain muscle cells encloses each tubule which is in form flask-shaped, *i. e.*, enlarged toward the blind end, with a constricted neck. These tubules open in two lateral groups in ths dorsal side of the cloaca immediately caudad of the openings of the oviducts; in *Triton* the number of tubules in each group varied from eight to fifteen; in *Salamandra* they were more numerous, being thirty or forty on each side. Large numbers of pigment cells occurred in the connective tissue surrounding the tubules.

The only other gland found at the cloaca of the female is the rudimentary abdominal gland of Heidenhain, found in *Triton*. Whether or not it is also present in *Salamandra* is unknown. As I shall have occasion to refer to a similarly situated gland in *Dicmyctylus* and certain other urodeles, it seems worth while to speak of this in some detail. Heidenhain describes it at some length. There are found in the entire circumference of the cloacal opening, upon the surface of the lips, papillæ of the integument. Microscopic examination showed that from the summits of these papillæ rudimentary gland tubules proceeded which have the same course as the abdominal gland tubules of the male and therefore must only be their homologs. From their origin they assume a strongly dorso-cephalic direction, traverse the cloacal lips, and, where they attain a considerable length, lie upon the ventral surface of the striated muscle which is situated on each side ventrad of the vertebræ. The length of the tubules varied considerably in the same individual. The lining cells were inconspicuous and presented no appreciable structure. The tubules were also very variable in number; as many as twelve paired and five unpaired tubules were found in some individuals, while in other examples there was no trace of them whatever. In regard to their homology with the abdominal gland tubules of the

male, I think it will be evident from a comparative study that the fact of their opening upon papillæ in the skin at the vent is insufficient to base a homology upon. Indeed, it seems futile to attempt to pronounce a homology between structures in male and female which possess in the two sexes clearly different functions, and are so variable that the gland may be well developed in the female in one genus of a family and totally wanting in another, even though they may occupy approximately the same position. For similar reasons, it does not seem to the writer that the spermathecas can be homologized with the pelvic gland of the male as has been so confidently done by Blanchard and Fischer. As has been said by Jordan, '93, the question is probably a barren one.

Dicmyctylus. (Fig. 7). Of the genera examined by me this is most nearly related to the European forms, and an approximation to the relations in those genera would be expected, and is found. The cloaca of the female is much less prominent than in the male; the vent is situated on rather a ridge-like elevation. Its lips are simple and no papillæ are present. The internal topography of the cloaca is also much simpler than in the male; the walls are thrown into low folds, not resembling, however, the ridges in the caudal portion of the cloaca of the male. In the dorsal side is a ridge which appears at the caudal end of the cloaca and steadily increases in height to within a short distance of the oviducal papillæ where it stops quite suddenly. As this ridge heightens, the cloacal cavity deepens dorsally, so that at the cephalic termination of the ridge there exists a deep dorsal depression in which soon appear the papillæ which bear the mouths of the oviducts. The ventral portion of the cloacal cavity simultaneously extends itself laterally and farther cephalad becomes the intestine. The bladder opens upon the ventral side of the cloaca, slightly cephalad of the oviducal papillæ.

The epithelium lining the cloaca was stratified in the caudal portion and columnar in the cephalic portion where it is composed of mucous cells. The stratified epithelium extends cephalad farthest on the dorsal side, even up to the area in which the

spermathecas open. These, in their structure and distribution seem to resemble closely those in the Salamandridæ. They are flask-shaped, the neck is very constricted and the diameter of the body of the cul-de-sac varies with the amount of distension caused by the zoösperms contained. The shape of the lining cells was also modified by the same cause; in the empty tubules, however, they were cubical. Each tubule is enclosed in a layer of plain muscular fibers which encircle it. These tubules open upon the lateral walls of the dorsal extension of the cavity which is divided into two parts by the mesal elevation. There were about twenty-five such tubules opening on each side. Cephalad of the termination of the mesal ridge, which is abrupt, the lateral groups meet at the meson and there occur five or six tubules which cannot be regarded as belonging to either group.

But two examples of this newt were examined by me; one taken March 10, the other in October. In both of them the spermathecas contained zoösperms. An examination of a more complete series of individuals taken at other seasons of the year would have been made had it not been already done by another. It should be done in an exhaustive study of the glands, for which, however, *Diemyctylus* is not as suitable a genus as *Amblystoma*. Jordan, 91, has said that he found zoösperms in the cloaca of the female "in nearly all the specimens examined between the first of May and the first of July," and in another place he says that zoösperms usually occur in the spermathecas of specimens taken in the autumn.

In addition to the spermathecas other tubules occur presumably homologous with the rudimentary tubules described by Heidenhain as representing the male abdominal gland. In *Diemyctylus*, however, they are clearly not rudimentary. The openings of the tubules occur upon the epidermis of the skin at the edge of the vent in its entire circumference. From their openings the tubules assume a dorsal, and generally, also, a cephalic direction on each side of the cloacal cavity. The more cephalic and also most mesal tubules do not extend dorsad, but directly cephalad and thus lie upon the ventral side of the cloaca. The gland

mass which will be spoken of as the "ventral gland," to avoid homologies, lies upon the ventral and lateral sides of the cloaca. A duct and a secreting portion of each tubule might be recognized; the former was constricted and its cells flattened; the secreting cells were columnar with their nuclei situated in the basal end. The cell itself appeared granular. This description applied only to the condition existing in the *Diemyctylus* taken in the spring; in the fall individual the cells were much smaller and the entire appearance was as of a gland of less functional activity, or one recovering from a state of exhaustion. Undoubtedly the latter was the case as what will be said subsequently of the seasonal variation in *Amblystoma* would indicate.

To compare *Diemyctylus* with the Salamandridæ a close approximation to relations there is seen. In the latter only the paired spermathecas would appear to exist. In the number of tubules, *Diemyctylus* is intermediate between the genus *Salamandra* and the Tritons. Wiedersheim mentions both the spermathecas and the ventral gland in *Salamandrina*.* Evidently that genus approaches *Diemyctylus* closely.

Necturus.** (Figs. 1–6). As in *Diemyctylus* the cloaca is much less prominent in the female *Necturus* than in the male, due to the weaker development of the glands. There are no cloacal papillæ and the lips of the vent are smooth. In the configuration of the cloaca *Necturus* differs somewhat from *Diemyctylus*. In the cephalic portion the oviducal papillæ, which are very large, project caudad and ventrad from the dorsal wall; caudad there is in the dorsal side a well-marked depression which extends almost to the caudal limit of the cloacal cavity. The epithelium of the cephalic portion of the cloaca and the dorsal depression is formed of mucous cells. Upon the inside of the lips of the cloaca this

*"Diese (Cloake) ist beim Weibchen von einem Kranz kleiner, schlauchförmiger Drüsen umgeben, welche in den die Spalte begrenzenden Lippen gelegen sind, und erst beim Auseinanderziehen der letzteren deutlich zum Vorschein kommen."

**Prof. Wilder has permitted me to examine some drawings of the cloacal cavity and papillæ of *Necturus* that were made in his laboratory in 1874 by Prof. W. S. Barnard.

merges into a stratified form of epithelium, which insensibly grades into the epidermis in which upon the cloacal lips the characteristic clavate cells are absent.

In the depression in the dorsal side of the cloaca there may be detected by the naked eye some forty or more orifices; these are the mouths of convoluted tubules which constitute the bulk of a gland mass of considerable size situated upon the dorsal side of the cloaca. (Fig. 2). These tubules were of large caliber and lined with tall columnar cells which presented the characteristic appearance of charged mucous cells. The nucleus was situated in the base of the cell the ectal portion of which was granular. There was no constriction of the tubule or differentiation of the epithelium to constitute a neck, but the columnar cells passed directly into the mucous epithelium of the cloaca, with whose cells they appeared identical in structure.

In addition to these large tubules there open upon the epithelium of the dorsal side of the cloaca in or at the edge of the depression other tubules of an entirely different appearance, the spermathecas. We find in them a repetition of the structure in *Diemyctylus*. They are flask-shaped, lined with cubical or low columnar cells and open upon the surface by a very constricted neck. Over forty of these were counted; they occur scattered among the other tubules over an area extending from just caudad of the mouths of the oviducts to the caudal limit of the depression. These almost invariably contained zoösperms. In addition to them other tubules of an entirely similar appearance open by the characteristic constricted neck into the free ends of the large convoluted tubules first mentioned. The cells lining them resembled in form and appearance those lining the tubules which I have just spoken of as the spermathecas, and were easily distinguishable from the taller granular cells of the convoluted tubules. Zoösperms were occasionally, though only occasionally, to be observed in these appendages to the larger tubules and they must likewise be considered spermathecas.

Six *Necturi* were examined and zoösperms were abundantly found in all in the spermathecas which opened on the cloacal epi-

thelium and rarely in those which opened into the large tubules. All were taken in the late fall or winter. It is unfortunate that it was impossible to examine any in which it was evident mating had occurred and the eggs were yet in the ovary. As it is, because of lack of knowledge of the habits of the animal, it is impossible to determine whether the zoösperms in the spermathecas of the forms taken in the winter were acquired in a fall mating, without ovulation, as occurs in *Diemyctylus*, or were merely such as had been left over after ovulation in the spring or fall, if one occurs at that season of the year. There might be a marked difference in the state of functional activity of the two sets of spermathecas, and those which opened into the tubules might be in reality the more functional.

The long convoluted tubules first described resembled no other cloacal gland. Indeed the impression is strong that they may arise as tubular proliferations of the mucous cells of the cloacal epithelium surrounding the mouths of some of the spermathecas, which they thus carry down with them in their development. It is possible that an examination of young forms would throw light on their origin and significance.

The ventral gland (Fig. 1) is present and well developed in *Necturus*. Its mass lies upon the ventral side of the cloaca cephalad of the vent, and its tubules open upon the stratified epithelium within the edge of the cloacal opening surrounding its cephalic portion. The tubules are paired and are some sixty or more in number on each side. A duct and secreting portion are well marked, the former is constricted and lined with flattened cells, the latter is of a larger diameter and formed of very tall columnar cells which are crowded with globules and large granules in the specimens examined. Doubtless they become more or less exhausted at the time of ovulation and gradually regain the resting state during the year as seems to be the case in *Amblystoma*.

Amblystoma. (Figs. 10–15). A more complete series of specimens of *Amblystoma punctatum* were examined and a better idea could thus be obtained of the extent and significance of sea-

sonal changes in the cloacal glands, and more satisfactory examination of the spermathecas was also possible. Those examined comprised seven individuals: (1) two *Amblystomas* taken in the spring in which the eggs were still in the ovary and no mating had yet taken place, as ascertained by examination which showed entire absence of zoösperms; (2) one in which the eggs were yet in the ovary, but mating had occurred—the spermathecas and depression were full of zoösperms; (3) one in which ovulation had just taken place in the laboratory; further, there were examined two taken in July and one taken in the winter (January).

In this form the relations at the cloaca differ markedly from those in either of the two genera just described. The ventral gland is present and occupies much the same position as in *Necturus;* the spermathecas open upon the dorsal epithelium of the cloaca and in addition there is another group of tubules situated in the dorsal wall of the cloaca opening upon the epithelium farther caudad. The relations of the three groups is well shown by the sagittal section, Fig. 10, from which also a general idea may be obtained of the shape of the cloacal cavity. The oviducts open as usual upon papillæ situated in a depression in the dorsal side of the cloaca; farther ventrad is another depression, quite narrow, into which the greater number of the spermathecas open and around which they are grouped; a few open upon the epithelium just caudad of it. The epithelium lining this depression, and also the cloacal cavity for a short distance cephalad and caudad of it on both the dorsal and ventral sides, consists of a single layer of columnar cells. Ciliated cells were found upon the ventral wall opposite the depression and cephalad of it on the dorsal side of the cloaca. In neither place was the tract extensive and the ciliated cells were often separated by non-ciliated ones. No cilia appeared on cells lining the depression. The arrangement of the spermatheca tubules was quite compact about the depression from which they radiated on all sides with a more or less dorsal trend. Between them strands of plain muscle cells passed, forming a network and encircling the depression more or less completely.

The ventral gland tubules some distance within the vent opened upon the cloacal epithelium which was there composed of two or three layers of cells; farther ectad it thickens to grade insensibly into the epidermis upon the lips of the cloaca. The tubules open upon the ventral and also to a slight extent upon the lateral sides of the cloaca, and radiate cephalad and also somewhat dorsad so that the gland mass is situated on the ventral and to a slighter extent the lateral sides of the cloaca. Though the mouths of the tubules do not occur in lateral groups, as in *Necturus*, the gland mass itself is divided on the meson into two equal lateral portions.

The second group of gland tubules which I have designated as the dorsal gland, opens on the dorsal and lateral sides of the cloaca and the tubules extend dorsad and cephalad.

In structure these glands were of an entirely different appearance. In both, the tubules were composed of the customary duct and secreting portion. The cells of the ventral gland were very tall columnar cells (Fig. 11). The cell body was crowded with large granules which took a cytoplasmic stain readily. The cells of the dorsal glands are finely granular and stain less intensely, coloring a light pink where the ventral gland stains rose. This is the appearance in the spring specimens taken before ovulation when the glands are in a resting condition and fully charged. In the specimen after ovulation the glands, though not exhausted, were yet so nearly so that an idea could be obtained of the grosser changes in secretion. Fig. 12 shows a partially exhausted tubule; the tall columnar cells filled with granules have shrunken, the lumen of the tubules is greatly enlarged and filled with the mass of secretion. A more complete stage of exhaustion is shown in Fig. 13, where the cells have become almost flattened. A comparison of the two summer forms in which the ovaries were spent shows the gland cells in an exceedingly reduced condition, in many tubules consisting of little besides nucleus. Fig. 14 shows one of the more developed tubules in which a few granules occur in the cells which are apparently just recovering and beginning to elaborate the secretion which will swell the cell so enormously. In fact, these glands, as do the

cells of the oviduct, seem to prepare themselves yearly for their period of secretive activity at the time of ovulation. The secretion found in the tubules and the cloacal chamber stains deeply with eosin. Changes in the dorsal gland seem to correspond closely with those in the ventral. I believe that these glands secrete the substance which forms the gelatinous mass which binds together the eggs when laid in masses.

So exactly do the spermathecas resemble in form and structure the gland tubules that we naturally turn to see if seasonal variations may also be detected in them. In the spring individuals in which the eggs are yet in the ovary and mating has not yet taken place, the conditions are most favorable for determining whether or not they present an appearance of cells in a resting condition, which might be expected if they elaborate any secretion attractive to the zoösperms. However, the cells show no traces of a stored secretion, and compared with the cells in the spermathecas of the specimen in which the eggs had been laid, but which still contained some zoösperms, show no apparent differences. The spermathecas contained more or less granular or stringy matter as of some coagulated secretion, as in all the other forms in which the empty tubules were observed—*Necturus*, *Spelerpes*, *Plethodon*. The cells resembled exhausted mucous cells, appearing finely reticular and staining lightly.

Spelerpes. (Figs. 16–17). But a single individual of *Spelerpes bilineatus* was examined, which was taken October 7. The structure of the spermatheca, however, was so unique and so suggestive of the meaning of the single mesal spermatheca in this and the two genera to be discussed subsequently, that a description is given, although no zoösperms were contained in the spermatheca of this one individual.

The structure of the cloaca in its caudal portion presents no unusual features; proceeding cephalad from the caudal end of the cloacal opening, the cavity deepens gradually and its walls are thrown into small longitudinal folds. Slightly caudad of the cephalic end of the vent, a narrow dorsal depression makes its appearance which deepens gradually until it meets the mouth

of the common tube of the spermatheca as it may be termed, which extends cephalad (Fig. 17). As this depression increases, more lateral ones appear cutting out or bordering lips for the mesal one, thus producing the appearance of a dorsal elevation cut in two. Cephalad of the opening of the spermatheca these folds disappear and a mesal elevation appears which is soon divided by a deep and narrow depression.

The spermatheca first appears in a series of transections slightly cephalad of the caudal end of the cloaca, upon its dorsal side and at first only loosely joined to it by connective tissue. In structure it consists of a central common tube into which near its caudal end open twelve to fourteen tubules, flask-shaped, with the body lined with columnar cells and opening by means of a constricted neck—a repetition of the structure of the tubules in the genera before described. (Fig. 16). The cells lining the common tube and the tubules opening into it are columnar and identical in appearance in the two places; they stain but lightly, resembling the cells of the spermathecas of *Amblystoma*. The epithelium of the spermatheca extends cephalad beyond its mouth some little distance, covering the mesal elevation before it becomes divided and persisting on each side for a short distance after the mesal depression appears.

The cloacal epithelium in its caudal portion is a stratified one, and two or three cells thick, which extends cephalad almost to the opening of the spermatheca. Mucous cells first appear in the lateral depressions alluded to above and spread over the entire surface of the cloaca except where the epithelium of the spermatheca extends cephalad, from which, because of the stain taken, they are readily distinguished.

The common tube of the spermatheca was of uniform structure throughout, somewhat larger at its blind end. It was enclosed in a thick layer of plain muscle cells which also surrounded each tubule opening into it. The entire organ is enclosed by a dense layer of pigment cells which is especially concentrated about the tubules. (Fig. 16). When the cloaca is dissected off, because of its pigmented state the spermatheca appears as a black

sack. It is impossible to resist the impression that the organ in *Spelerpes*, and therefore in the two following genera, is not the equivalent of a single one of the many tubules of *Diemyctylus* which has become enlarged and branched, but presents an exaggeration of the condition in *Amblystoma*, and represents a tubular depression of the cloaca, into the end of which the clustered tubules open,—which in such forms as *Diemyctylus* open upon the cloacal epithelium directly,—and thus seems to constitute a connecting link between *Diemyctylus*, *Necturus* and *Amblystoma* on the one hand, and *Plethodon* and *Desmognathus* on the other. The tubules as well as the common tube contained more or less matter, granular or globular which might seem to indicate, as in *Amblystoma*, a secretive activity of the cells.

Opening upon the dorsal side of the cloaca, caudad of the mouth of the spermatheca, are some twelve tubules which were in a very undeveloped condition and were either rudimentary or in a state of inactivity or exhaustion. In some the cells consisted of little more than the nucleus, in others they were cubical and in all a lumen was present. Likewise, cephalad of the opening of the spermatheca, were six or seven short tubules of a rudimentary appearance. Whether these represented the dorsal cloacal gland or were spermatheca tubules which had become rudimentary from disuse—if the spermatheca were not in the first place modified tubules of the dorsal gland—is, of course, idle speculation. In the absence of a knowledge of the habits of the salamander or specimens taken at different times of the year, any conclusion is impossible.

The ventral gland is well developed; its tubules open upon the cloacal epithelium of each side some distance within the vent. They are all paired and are some twenty in number on each side. The more caudal tubules extend dorsad, those more cephalad lie upon the ventral side of the cloaca and there form a considerable gland mass. The location of the gland, therefore, is as in *Diemyctylus*. The cells of the tubules are columnar and resemble those in that genus.

Plethodon erythronotus. As in *Spelerpes*, the spermatheca in

this salamander is mesal though it differs much in structure. A feature peculiar to *Plethodon* among the forms examined is the presence of a large papilla-like elevation which projects caudad and ventrad from the dorsal wall of the cloaca in its caudal portion. Strands from the circular muscular coat of the cloaca project up into it, so that it is doubtless more or less contractile. Dorsad of its base the cloacal cavity extends cephalad a short distance as a cephalic recess.

Cephalad of this papilla there appears a dorsal elevation on which opens the spermatheca. As in *Spelerpes* this organ is sack-like in shape and highly pigmented and muscular. *Plethodon* differs, however, in that the common tube has a constricted neck, and instead of the twelve tubules opening into its blind end there are but four diverticula which do not show the characteristic structure possessed by the tubules in the previous forms, namely, a body and neck, though they are somewhat constricted at the opening into the common tube. The common tube and to a less degree each of the diverticula have an encircling muscular tunic. The circular muscular coat of the cloaca proper is well developed and sends bundles of cells up into the elevation on which the spermatheca opens and also into the spermatheca.

Cephalad of the spermatheca the dorsal ridge becomes thinner and finally disappears; the cloacal cavity extends itself laterally and dorsally as a depression into which open the oviducts from short papillæ, and the ureters. The epithelium of the cloaca in the caudal position is stratified, as before, and this extends farthest cephalad upon the dorsal elevation, to slightly caudad of the spermatheca. It is succeeded by a mucous epithelium, which lines the cephalic portion of the cloaca, first appearing caudad on its ventral side.

Plethodon glutinosus. (Figs. 18–19). This species when compared with *erythronotus* shows close resemblances with some marked variations. The curious tongue-like papilla in the caudal portion is well developed and the projection of the cavity beneath its base is considerable. The spermatheca is much as in *Plethodon erythronotus*; the diverticula are four in number and the com-

mon tube into which they empty opens upon the cloaca by a very constricted neck at the summit of the dorsal ridge (Fig. 19) as in *erythronotus*. A difference, however, consists in the fact that the common tube is lined with a stratified epithelium of flattened cells. The muscularity and pigmentation of the organ are also greater. Pigment is not confined to this region, but occurs in the tongue-like papillæ, and to a slight extent in the circular muscular coat of the cloaca. A most peculiar difference between the two species lies in the presence of cilia in the cloaca of *glutinosus*, and their entire absence so far as I can determine in *erythronotus*, the tissue being fixed in the same way in each. In *Amblystoma* a few scattered ciliated cells were found, but in *P. glutinosus* the area was extensive. As in *erythronotus* the caudal portion of the cloaca is lined with a stratified epithelium two or three cells deep, which extends cephalad upon the dorsal elevation to a point slightly cephalad of the mouth of the spermatheca. In the depression on each side of this elevation ciliated cells first appear; they spread laterad and ventrad until the area encircles the entire cloaca.

Immediately cephalad of the opening of the spermatheca and cutting into the base of the elevation (Fig. 19), the cloacal cavity extends, laterad and dorsad as in *erythronotus*, constituting the depression into which open the ureters and oviducts, the latter upon very small papillæ, if any. These depressions are clothed with large mucous cells, which spread laterad and ventrad to clothe the entire circumference of the cavity.

Of *erythronotus* were examined specimens taken in the summer (2), fall (1) and spring (4), and only in three in the spring, taken April 15th and May 2d, were zoösperms found. Two individuals of *P. glutinosus* were examined, and in neither were zoösperms contained in the spermatheca. There is no doubt but that were a larger number examined, zoösperms would be found as in *erythronotus*.

Geotriton, the only European genus of this family, was examined by Fischer, and a single mesal spermatheca, as in *Plethodon*, was found, though in the specimens examined by him no zoösperms were contained.

In those individuals in which no zoösperms were contained, the spermatheca held, as in *Spelerpes*, granular and reticulated matter, as if of coagulated secretion, and in some instances the free ends of the cells appeared flagellate or threaded.

Desmognathus (Figs. 8 and 9). The relations are much as in *Plethodon*. An elevation appears upon the dorsal side of the cloaca, and upon this, as in *Plethodon*, the spermatheca opens (Fig. 9). This is very well developed, consisting of a common tube with five or six large diverticula, and opens by constricted neck with flattened cells. The muscular sheath of the common tube and its diverticula was strong. Both the diverticula and the common tube were lined with low columnar cells, presenting no marked structural features. The pigment surrounding the organ was very abundant, and rendered it easily recognizable on dissecting out the cloaca for sectioning.

The general configuration of the cloacal chamber was much as in *Plethodon*, save that no tongue-like papillæ existed. The elevation in the dorsal wall, already referred to so often, was present. The stratified epithelium of the caudal portion of the cloaca extends cephalad upon it up to the mouth of the spermatheca. Mucous cells first appear in the depression on each side of the elevation, whence they spread ventrad to completely clothe the cloacal wall. Cephalad of the spermatheca the elevation disappears and the cloacal cavity assumes a tubular form. The oviducts and ureters open relatively farther cephalad than in *Plethodon*, the former upon the summits of low papillæ.

Specimens taken in the summer, fall, winter and spring were examined, and in all the spermathecas were well filled. Though a confirmation from the meager facts can not be had, it seems probable that such a difference in the presence of zoösperms in *Plethodon* and *Diemyctylus* has an accompanying difference in habits of mating which would explain it. Though doubtless merely indicating the way in which the zoösperms entered the spermatheca, the fact that in the individuals examined they almost invariably lay with their heads toward the cells of the spermatheca, might indicate an attractive principle secreted by the cells.

Neither in *Plethodon* or *Desmognathus* were ventral or dorsal cloacal glands present.

CONCLUDING REMARKS.

From the foregoing it is seen that in the six genera examined, including urodeles of widely different habits, functional spermathecas are to be found, though with a great variation in structure. Indeed, in *Diemyctylus*, *Amblystoma* and *Necturus* they may more strictly be spoken of as individual tubules which function as spermathecas, possibly gland tubules, as they are termed by Gegenbaur and Claus, so exactly do they compare with the tubules which constitute the other cloacal glands; and it is first in *Amblystoma* that there appears in the arrangement of the tubules around a depression the suggestion of the unity of structure found in the remaining three genera, *Spelerpes*, *Plethodon* and *Desmognathus*, where there is a single mesal spermatheca.

All discussion heretofore has been purely anatomical, and only incidentally has the function and physiological action been alluded to. The question may be fairly put, how far are the spermathecas of real importance to the salamander, and have they in every case a rôle to play in the process of fertilization, or are the zoösperms contained only used in certain cases? Our ignorance of the habits of all but one of the genera forbids an estimate, but as far as more isolated observations afford an answer, it is that their functional use is complete; in other words, that the zoösperms taken up by the female are stored away in these tubules and are expelled from them when needed for the fertilization of the egg. In some forms their functional use is great. Both Czermak and Siebold have shown that the female of the viviparous *Salamandra atra*, with but one mating in the spring, yet gives birth to its young several times during the year, and Zeller considers it undoubted that the same is true for *Salamandra maculosa*.* For these viviparous forms, therefore, the

* "Ja, es ist dafür der thatsächliche Beweis durch die schon zum öftern gemachte Beobachtung erbracht, dass isolirt gehaltene Weibchen [*Salamandra maculosa*] nach ein und sogar nach zweijähriger Gefangenschaft eine kleine Anzahl von Larven geboren haben." Zeller, '90, p. 595.

spermatheca is of the utmost importance. In *Triton* (*alpestris* and *tæniatus*) Zeller regards it as exceedingly probable that in certain cases fertile eggs may be laid in the spring without a previous mating that spring, though this is unusual. In this form, according to him, the zoösperms received from a mating will fertilize one hundred or more eggs, which are laid within eight to fourteen days, when another mating occurs. With the axolotl the proceedure is more rapid; ovulation generally begins the following night and within thirty-six or forty hours 300 to 600 eggs have been deposited; occasionally the number reaches 800 to 1,000 (Zeller): in the aquarium two ovulations may occur in the same year, but the second is preceded by a fresh mating. The observations of Zeller, Gage and Jordan, especially the last, make us more familiar with the phenomena of fertilization and ovulation in *Diemyctylus* than in any other form. Jordan says: "It is probable that for a single individual the egg-laying season lasts for at least seven or eight weeks. The longest time over which I have actually observed the laying of a single individual to extend is four weeks, but in this case the ovaries still retained large pigmented eggs, and under perfectly normal conditions egg-laying would undoubtedly have continued for some time longer." The largest number of eggs laid under observation was ninety-six, and ovulation lasted nineteen days after separation from the male. An autumnal mating in *Diemyctylus* was first observed to occur by Gage, and confirmation of this has been given by Jordan, '92. It is possible, therefore, in certain cases, that the laying of fertile eggs in the spring may proceed without a previous spring mating.

But a slight clue of the time and manner of ovulation can be gained from a study of the spermathecas, since it can never be determined whether the zoösperms present are an accession of a recent mating or are those remaining after a sufficient number has been used in the fertilization of the ova. It would appear probable that in *Amblystoma* there is but one mating in the spring followed by ovulation. Of the habits of *Necturus* at present no definite conclusion can be drawn. The fact that zoösperms were

universally found in *Desmognathus* in the spring, summer, fall and winter, and found only in the spring in *Plethodon* suggests some difference in habit, though too few individuals were examined to afford more than a suggestion. Sherwood says of *Desmognathus*: "I think there are two broods annually, as I have found eggs from July to October and have seen very small larvæ as late as November 30." He found eggs of *Plethodon* and *Spelerpes* October 25th. Three specimens of *Plethodon erythronotus*, taken by me between the 15th of April and the 2nd of May, all had the spermathecas well filled, and there is therefore a suggestion of the possibility of two broods in this form. Mere speculation, however, is idle and direct observation of the habits of these forms must be awaited.

Jordan, from his careful study of *Diemyctylus*, concludes that fertilization in that genus takes place in the cloaca as the ovum passes through to be extruded; his words are: "The fertilization of the egg takes place just before the egg is extruded. The spermatozoa, which have long been waiting in the tubes of the receptaculum seminis, are either attracted from their resting places by the passing egg or forced out by contraction of the surrounding muscles. I have made repeated and careful search for spermatozoa in the oviducts, but have never succeeded in finding one. Neither have I ever found in sections any indication that spermatozoa enter oviduct eggs, although eggs often lie for some time in the mouth of the oviducts. Fertilization, then, would seem to take place only after the egg has left the oviduct and passed into the cloaca." I would say that I also have never seen zoösperms in the oviducts of any species sectioned by me, in almost all of which the lower portion of the oviducts was examined. However, in the viviparous *Salamandra artra* and *maculosa* fertilization of the egg must occur in the oviduct since development takes place there. Spallanzani (as quoted by Zeller) stated that eggs removed by him from the oviducts of the female *Triton* proved fertile and developed. Robin, also, said that examination of the female *Triton* at the time of ovulation discovered zoösperms in the cloaca and "three or four millimeters within the oviducts."

There would seem, then, to be a variation in the different forms.

The walls of the cloaca are largely formed of plain muscular tissue which surrounds the vent and strands of muscle cells interdigitate with the gland and spermatheca tubules, and it is probable that in fertilization the zoösperms are forcibly expelled by muscular contraction.* In the forms in which there is a mesal spermatheca, the whole organ and its common tube are encircled by a thick layer of muscle cells, and that such is the case is especially evident here.

"The question as to how the spermatozoa find their way into these snug resting-places is one of considerable interest. Why should they enter these small ducts and there lie dormant, in preference to passing *en masse* up the oviducts, or to entering the alimentary canal, or even to issuing from the mouth of the cloaca? It appears to be probable that the explanation lies in what Pfeffer has called 'positive chemotaxis.' Pfeffer found, as is well known, that certain chemical substances, as malic acid, attract spermatozoa (positive chemotaxis), and that others, as chloroform, repel them (negative chemotaxis). For example, the mucilage in the central canal of the archegonia of *Pteris* contains a trace of malic acid, and Pfeffer has shown that this amount is sufficient to attract spermatozoa to the mouth of the canal. A similar explanation has been given by some bacteriologists to account for the gathering of leucocytes at inflammatory foci. It is supposed that the leucocytes have been drawn thither in virtue of their chemotactic properties which were brought into play by the metabolic bacterial poisons, or, as now seems more likely, by the freed albuminoid constituents of the bacterial cell.

"It seems highly probable that the pelvic gland of the female

*"The explanation of the dropping of non-fertilized eggs seems to be that in females with full oviducts, an egg is occasionally pressed into the cloaca by the mere elasticity of the oviduct walls and without the special cognizance of the newt. This egg then passes out like so much excreta without the performance of a voluntary act of oviposition. The fact that these eggs are, for the most part, unfertilized indicates an expulsion of spermatozoa from the receptacle during the performance of egg-laying." Jordan, '95, p. 309.

newt may secrete a substance—proteid or otherwise—with a positively chemotactic effect, and thus draw the spermatozoa into its ducts. At all events, such a supposition may serve for a provisional hypothesis." (Jordan, '91, p. 266.)

The question set forth in the above quotation is indeed difficult to answer. At first sight one would seem compelled to assume such attractive secretion by the cells of the spermatheca. Indeed, examination of the tubules in *Amblystoma* or other forms when they are free from zoösperms, shows clearly that some secretion is given off by the cells of the tubules, though of its nature and significance nothing can be said. It does not seem at all impossible, however, that the entrance of the zoösperms may be due solely to their own activity assisted by muscular contractions of the cloaca and spermatheca.

The great abundance of pigment cells surrounding the spermatheca tubules in all forms except *Necturus* and *Amblystoma* has been noted in the discussion of each form. Though, therefore, it is not a constant accompaniment of the spermatheca, the great abundance of it surrounding the organ in the forms in which it is most highly developed cannot but attract attention and arouse speculation. When the zoösperm enters the pigmented ovum it has been observed to exert an attractive influence upon the pigment particles, which cluster to meet its entrance and follow it as a pigment trail as it penetrates deeper (see Jordan, '93, p. 317). Were pigment cells constantly found surrounding the tubules, it might be suggested that the attraction exerted by the zoösperms was potent here, hence the pigmentation; or that the attraction between pigment and the zoösperms was mutual and the pigment enticed them into their resting places. Pigment occurs surrounding other gland tubules, and in other regions of the cloaca, though less plentifully, so that its presence is doubtless due to something other than an attraction exerted by the zoösperms, though this might yet account for its greater concentration at that point.

Blanchard regarded the tubules which have been seen to function as reservoirs for the zoösperms as homologous with the

pelvic gland of the male, as likewise does Fischer, and Jordan, though very guardedly. As before stated, I do not believe a homology can safely be declared between any of the cloacal glands of the male and female, which are so evidently dependent on their function for their existence, which is again clearly different in the two sexes. In regard to the spermathecas, difficulties occur in *Amblystoma* and *Necturus* in the presence of other tubules in the dorsal wall of the cloaca, which are again so different in the two genera. With Jordan, I regard the question as a barren one.

Besides the spermatheca tubules, there occurs in all, except *Desmognathus* and *Plethodon*, a group of gland tubules upon the ventral side of the cloaca, which has been termed the ventral cloacal gland. In *Amblystoma*, *Spelerpes* and *Necturus* there is a second group upon the dorsal side of the cloaca, which in *Amblystoma* I have termed the dorsal gland. The tubules in *Necturus* do not seem comparable or homologous. These glands are clearly accessory genital glands, and their secretion is without doubt of use in ovulation, though the function can not be stated more definitely as yet; possibly it serves as a cement to cause the eggs to adhere to that on which they are laid, or to each other. The ventral gland is best developed in *Necturus* and *Amblystoma*, least so in *Diemyctylus* and *Spelerpes*. It may be pointed out that the two forms in which the ventral gland is wanting are such as lay their eggs on land.

METHODS.

Since it is customary to give an account of the methods employed, the following is added, though no attempt was made to develop them. The examination of the cloaca was accomplished by means of serial sections made transverse to the long axis of the body, to which were added in *Plethodon*, *Desmognathus* and *Amblystoma*, series cut sagittally. The cloaca was dissected off and placed for twenty-four hours in Fish's mixture. (Formula: 50 per cent. alcohol, 1000 cc.; mercuric chlorid, 5 grams; picric acid, 1 gram; glacial acetic acid, 10 cc.) It was

then washed in 50 per cent. alcohol one day, and passed through successively 70, 82 and 95 per cent. alcohols, ether-alcohol (equal parts), remaining one day in each. It was placed in 1½ or 2 per cent. collodion for two days, and 6 per cent. collodion for three days, and imbedded. The collodion was hardened in chloroform and cleared in Fish's castor-thyme-oil mixture, in which the sections were cut. They were arranged in serial order on the knife, from which they were removed by tissue paper, placed upon the slide; all oil possible was absorbed with tissue paper and the sections secured by melting the collodion with a few drops of ether-alcohol. A few minutes (5 to 15) in 95 per cent. alcohol sufficed to remove all the oil when they were treated as usual. Hæmatoxylin (Gage's) with eosin, erythrosin or picric alcohol, as a counter-stain, were employed. Vasale's clear (Xylene three parts, carbolic acid one part), was used. This was supplemented by teasing fresh spermathecas upon the slide to detect the living zoösperms.

SUMMARY.

1. In the genera *Necturus*, *Amblystoma*, *Diemyctylus*, *Plethodon* and *Desmognathus* spermathecas are found in the dorsal wall of the cloaca of the female, containing zoösperms. Internal fertilization is therefore proven for these forms.

A spermatheca occurs in *Spelerpes*; in the single specimen examined (taken in the fall) no zoösperms were contained.

In *Necturus*, *Diemyctylus* and *Amblystoma* there are several tubules or spermathecas opening upon the cloacal epithelium, which serve as reservoirs for the semen.

In *Diemyctylus*, *Plethodon* and *Spelerpes* there is a single mesal spermatheca.

The condition in *Spelerpes* would seem to indicate that the organ in these latter genera equals the group of tubules found in the first genera plus an exaggerated and modified depression of the cloacal epithelium, such as occurs in *Amblystoma*.

2. No gland-like structures in addition to the spermatheca occur in the female of *Plethodon* and *Desmognathus*.

3. In all the remaining genera a ventral cloacal gland is present.

4. In *Amblystoma*, *Spelerpes* and *Necturus*, in addition to the spermatheca tubules other tubules occur on the dorsal side of the cloaca.

5. The secretion of the cloacal glands is employed at the time of ovulation.

6. The three glands of the male recognized in the *Triton*, the cloacal, abdominal and pelvic, occur and are well developed in the five genera examined. This suggests that by all of these spermatophores are deposited.

7. A résumé of the literature and the foregoing facts points to a uniform mode of mating and fertilization in all urodeles.

8. Dorsal and ventral ciliated tracts occur in the male of all the genera examined (five). Cilia in the cloaca of the female were detected only in *Amblystoma* and *Plethodon glutinosus*, where the tract was not as extensive as in the male.

LIST OF PAPERS REFERRED TO.

'85. Balfour. F. M.—A treatise on comparative embryology. Vol II., pp. 120–121. 1885.

'79. Bedriaga, J. von—Beiträge zur Kenntniss des Rippenmolches. *Verhandl. der Kais. Gesellsch. der Wiss. zu Moskau*, p. 179. 1879.

'82. Bedriaga, J. von—Ueber die Begattung bei einigen geschwänzten Amphibien. *Zoologischer Anzeiger*, Vol. V., pp. 265–268, & 357–359.

'81. Blanchard, R.—Sur les glandes cloacale et pelvienne et sur la Papille clocale des Batraciens et des Urodèles. *Zool. Anz.*, Vol. IV., pp. 9 & 34.

'83. Chauvin. Marie von—Die Art der Fortpflanzung des Proteus anguineus. *Zeitsch f. wiss. Zool.*, Vol. XXXVIII., pp. 671–685. 1883.

'79. Clark, S. F.—The development of Amblystoma punctatum. Part 1. External. *Studies from the Biol. Lab'y of Johns Hopkins Univ.*, Vol. I., pp. 105–125

'82. Claus. Grundzüge der Zoologie. Vol. II. Translated by Sedgewick. London, 1884.

'89. Cope, E. D.—The Batrachia of North America. Bull. No. 34 *U. S. Nat'l Museum.* 1889. Washington, D. C.

'43. Czermak, J. J.—Beiträge zur Anatomie und Physiologie des schwarzen Salamanders. *Med. Jahrb. des Oesterr. Staates*, Vol. XLV., p. 8.

'95. Eycleshymer, A. C.—The early development of Amblystoma punctatum, with some observations on some other vertebrata. *Jour. Morph.* Vol. X., pp. 343–418.

'93. Fick, R.—Ueber die Reifung und Befruchtung des Axolotleies. *Zeitschrift für wissenschaftliche Zoologie*, Vol. LVI., pp. 529–614. 1893.

'91. Fischer, G.—Beiträge zur Kenntniss des Geotriton fuscus. *Verhandl. physik.-med. Gesellsch., Würzburg*, Vol. XXV. 1891.

'93. Fish, P. A.—A new clearer for collodionized objects. *Proc. Amer. Micr. Soc.*, Vol. XV., pp. 1–4.

'91. Gage, S. H.—Life-history of the vermillion-spotted newt (Diemyctylus viridescens, Raf.). *Am. Nat.*, Dec. 1891, pp. 1084–1110.

'80. Gasco, F.—Gli amori del Tritone alpestre. Genova, 1880.

'81. Gasco, F.—Les amours des Axolotls. *Zool. Anz.* Vol. IV., pp. 313, 334. *Bull. Soc. Zool., France*, 1881, pp. 151–162.

'78. Gegenbaur, Carl.—Elements of Comparative Anatomy. Transl. by F. J. Bell, London. 1878.

'90. Heidenhain, M.—Beiträge zur Kentniss der Topographie und Histologie der Kloaka und ihrer drüsigen Adnexa bei den einheimischen Tritonen. *Arch f. mikr. Anat.*, Vol. XXXV., pp. 173–274.

'78. Hoffmann, C. K.—Bronn's Klassen und Ordnungen des Thierreiches. Vol. VI., part 2, Amphibien.

'91. Jordan, E. O.—The Spermatophores of Diemyctylus. *Journal of Morphology*, Vol V., pp. 263–270.

'93. Jordan, E. O.—The habits and development of the newt (Diemyctylus viridescens). *Jour. Morph.*, Vol. VIII., 1893, pp. 269–352.

'78. Lataste, F.—Sur l'accouplement chez les batraciens urodèles. *Revue internat. des sci.*, No. 42. Paris. 1878.

'53. Leydig, F.—Anatomish-histologische Untersuchungen über Fische und Reptilien. 1853.

'20. Rathke. — Beiträge zur Geschichte der Thierwelt. Erste Abtheilung. *Neueste Schriften der naturf. Gesellsch.* in Danzig, Vol. I., Danzig, 1820.

'74. Robin, Ch.—Observations sur la fécondation des urodèles. *Jour. de l'anat. et de la Physiol. norm. et path. de l'homme et des animaux.* Vol. X, pp. 376–390. Also, *Annals & Mag. Nat. Hist.*, Vol. XIV., p. 96.

'21. Rusconi, D. M.—Amours des Salamandres aquatiques, 4to. Milan. 1821.

'54. Rusconi, D. M.—Histoire naturelle development et Metamorphose de la Salamandre terrostere. Pavia, 1854.

'95. Sherwood, W. L.—The salamanders found in the vicinity of New York City, with notes upon extra-limital or allied species. *Proc. Linnean Soc. of New York*, No. 7, 1895, pp. 21–37.

'58. Siebold, C. Th. von—Ueber das Receptaculum seminis der weiblichen Urodelen. *Zeitsch. f. wiss. Zool.*, Vol. IX, p. 463, 1858.

1785. Spallanzani, L.—Experiences pour servir à l'histoire de la génération des animaux et des plantes; avec une ebauche de l'histoire des êtres organisés avant leur fécondation. Par. J. Senebier, Geneve, 1785.

'91. Stieda, Alfred—Ueber die Kloaka und das Receptaculum seminis der weiblichen Tritonen. *Inaug. Dissert. Königsberg*, July, 1891.

'75. Wiedersheim, R.—Salamandrina perspicillata und Geotriton fuscus. Genua, 1875.

'90 Zeller, Ernest—Ueber die Befruchtung bei den Urodelen. *Zeitsch. f. wiss. Zool.*, Vol. XLIX., pp. 583–602. 1890.

'91. Zeller, Ernest—Berichtigung, betreffend der Samenaufnahme der weiblichen Tritonen. *Zeitsch. f. wiss. Zool.*, Vol. LI, pp. 737–741.

EXPLANATION OF THE PLATES.

All figures were outlined by means of the Abbe camera lucida and details were added free hand. The approximate degree of magnification is given in the explanation of the figures which are placed with the dorsal side uppermost.

PLATE I.

FIG. 1. Cloaca of *Necturus*. A transection just cephalad of the vent. The ventral gland tubules are shown opening upon the ventral side. On the dorsal side are cut spermathecas and convoluted tubules, some of the former are shown opening upon the epithelium. × 8.

v. gd.=ventral gland.
c. t.=convoluted tubules.
sp.=spermatheca.
c.=cloacal cavity.

FIG. 2. Cloaca of *Necturus*. A transection farther cephalad showing the opening of the large convoluted tubules and spermathecas into the dorsal depression. A fragment of the ventral gland is seen. × 8.

v. gd.=ventral gland.
c. t.=convoluted tubules.
sp.=spermatheca.
c.=cloacal cavity.

FIG. 3. A spermatheca opening into a convoluted tubule. × 48.

c. t.=convoluted tubule.
sp.=spermatheca.

FIG. 4. Isolated cells from a convoluted tubule. × 312.

n.=nucleus.

FIG. 5. Isolated secreting cells from a ventral gland tubule. × 312.

n.=nucleus.

PLATE I.

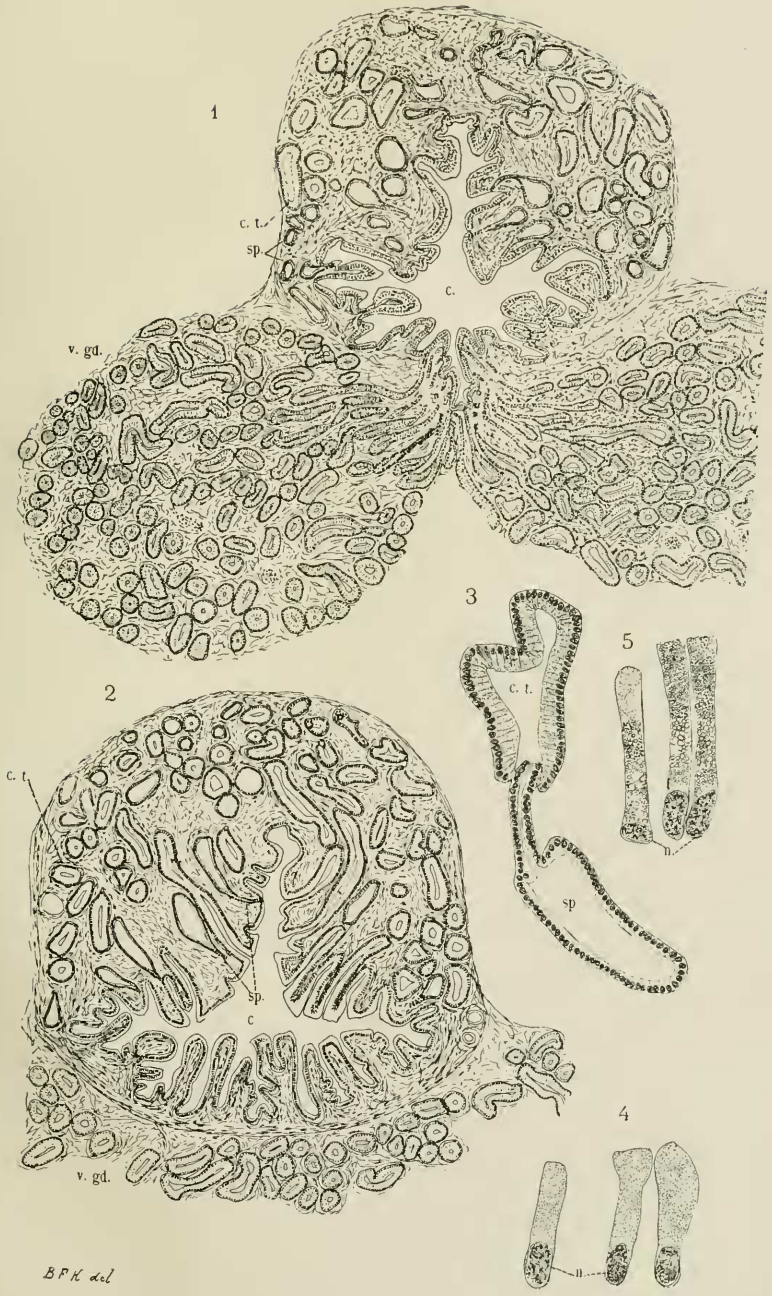

PLATE II.

FIG 6. Cloaca of *Necturus*. Transection yet farther cephalad through the oviducal papillæ. The mass of tubules upon the dorsal side is shown, some of which are spermathecas, others convoluted tubules. The neck of the bladder is also transected. × 8.

sp.=spermatheca.
c.=cloacal cavity.
c. t.=convoluted tubules.
ov.=oviducts.

FIG. 7. Cloaca of *Diemyctylus*. Transection a short distance cephalad of the vent. The spermathecas are seen cut at different levels. The bulk of the ventral gland lies farther caudad and only a few tubules were transected. × 26.

ep.=epidermis.
c.=cloacal cavity.
sp =spermatheca.
v. gd.=ventral gland.
M.=striated muscle.

FIG. 8. *Desmognathus fusca*. A transection through the cloaca and spermatheca. The zoösperms are shown in the diverticula of the latter. A prominent dorsal fold is present on which farther cephalad the spermatheca opens. × 26.

sp.=spermatheca.
c.=cloacal cavity.
ep.=epidermis.
z.=zoösperms.

FIG. 9. *Desmognathus fusca*. A transection through the cloaca farther cephalad, showing the common tube of the spermatheca in the dorsal elevation just caudad of its opening. × 26.

ep.=epidermis.
c.=cloacal cavity.
c. s. t.=common spermathecal tube.
K.=Kidney.
M.=striated muscle.

PLATE II.